COTTON EFFECTS IN THE β-LACTOGLOBULINS

Serge N. Timasheff and Robert Townend

Eastern Regional Research Laboratory[1]
Philadelphia, Pennsylvania, 19118

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Bovine β-lactoglobulin has been known for a long time for its unusual optical rotatory properties: the native protein has one of the lowest values of $[\alpha]_D$ reported for a globular protein, while denaturation results in one of the largest increases in levorotation observed in proteins (Groves et al., 1951; Scnellman, 1958). In more recent studies of the optical rotation dispersion (ORD) of this protein, it has been found that both Moffitt-Yang (1956) parameters, a_o and b_o, are small and negative (Tanford et al., 1960; Herskovits et al., 1964), leading to the proposals that β-lactoglobulin either has almost equal amounts of right-handed and left-handed helices (Urnes and Doty, 1961; Bell and McKenzie, 1964) or that it contains ordered structures other than α-helical (Tanford et al., 1960; Herskovits et al., 1964), e.g. the β-structure. A detailed examination of the a_o and b_o parameters of the three genetic variants of this protein, β-A, β-B and β-C (Herskovits et al., 1964), has revealed that b_o hardly varies between pH 1 and 12, its values lying between -60 and -85 for all three proteins, while a_o changes strongly with changes of pH and temperature; these changes follow quantitatively both the known changes in the state of aggregation and the reversible conformational transitions which exist in all three β-lactoglobulins in the pH intervals of 4 to 6 (Timasheff, 1965) and

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

6.5 to 9 (Tanford et al., 1959). Above pH 9, irreversible time-dependent denaturation sets in and a_o assumes increasingly more negative values down to around -620 at pH 12. In view of this abnormal variation of the two Moffitt-Yang parameters, a detailed examination of Cotton effects in all three genetic variants has been carried out using a Cary Model 60 spectropolarimeter[2] as a function of pH from pH 2 to 11.5 and of the methanol content of the solvent (from 20% to 100%), as well as of 8 M urea. The disulfide cleaved S-sulfonated derivative has also been examined in these solvents. Detailed results, some of which are summarized in the present communication, will be reported elsewhere.

All three bovine β-lactoglobulins exhibit Cotton effects in two wavelength regions, one between 280 and 300 mμ and the other between 195 and 240 mμ. Typical curves are shown in Figures 1 and 2. In the high wavelength region the native species of all three genetic variants have a small double Cotton effect with maxima at 291 and 284 mμ and minima at 296 and 286 mμ. These are essentially identical in all three proteins and are visible from pH 2 to 8.8. The position of this Cotton effect suggests the involvement of tryptophan residues (Kronman et al., 1965) in an ordered structure, although tyrosines (Fasman et al., 1964) can not be excluded. This ordered structure persists through the reversible conformational changes below pH 8.8, but is destroyed by denaturation by alkali, urea or methanol. The presence of this effect furthermore accounts for the fact that Moffitt-Yang plots of β-lactoglobulin ORD data have their lowest wavelength points consistently (Schellman, 1958; Tanford et al., 1960; Herskovits et al., 1964) lying away from the straight line drawn through points above 350 mμ.

[2]It is not implied the U. S. department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

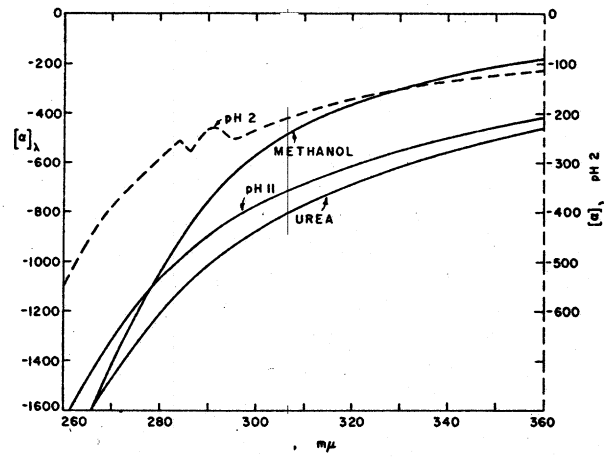

Fig. 1. Optical rotation dispersion of β-lactoglobulin A in the 260-360 mμ region. Protein concentrations are 2.13 g/1 in all four cases. The pH 2.0 curve refers to the right ordinate, the other curves (pH 11.3, methanol + 0.01 M HCl and 8 M urea in 0.01 M HCl) refer to the left ordinate.

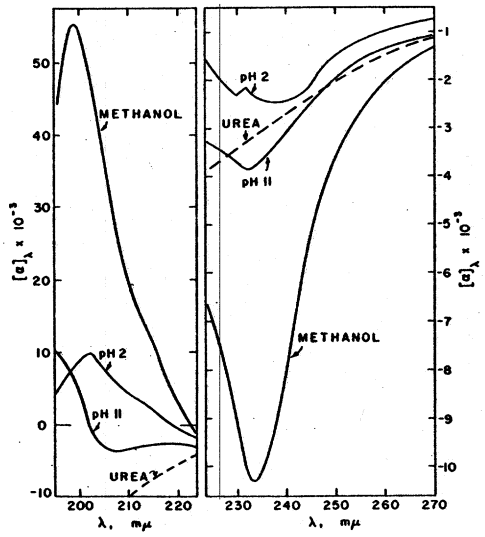

Fig. 2. Optical rotation dispersion of β-lactoglobulins in the 195 to 270 mμ region. The curves are for β-A in methanol + 0.01 M HCl, 0.24 g/1; β-A in 8 M urea + 0.01 M HCl, 0.27 g/1; β-C at pH 2.1, 0.21 g/1; β-C at pH 11.2, 0.21 g/1.

In the low wavelength region, the ORD curves become somewhat more complicated. In the native state, the ORD curves of all three proteins are characterized by a shallow trough ($m' \sim -1,800°$) with two minima at 230 and 238 mμ, separated by a small maximum at 233 mμ (Figure 2, pH 2). That this detailed shape of the ORD curve is real is evidenced by the fact that all three variants give the identical detailed pattern which is unchanged from pH 2 to 6. In the same pH range, all ORD curves pass through a maximum at 200-202 mμ with $m' \sim 8,500°$. When the pH is raised, the 230-240 trough attains increasingly more negative values, while the 200 mμ maximum shifts to lower wavelengths. At pH 11-11.5, where the proteins are irreversibly denatured, the ORD curves (Figure 2, pH 11) assume a shape with a minimum at 232 mμ ($m' \sim -3,500°$) and a second broad minimum at $\sim$ 208 mμ, while no maximum can be seen yet down to 195 mμ. 8 M urea, on the other hand, leads to the disappearance of all Cotton effects between 210 and 300 mμ.

Addition of methanol to a pH 2 solution of β-lactoglobulin produces few changes in the ORD curve up to 30% by volume. At 40%, the 280 mμ Cotton effect disappears, and in the low wavelength region the curve assumes the shape normally assigned to α-helical structures (Blout et al., 1962) with a minimum at 234 mμ, a maximum at 199 mμ and a positive shoulder in the 210-215 mμ region, a situation which is not surprising in view of the studies of Tanford and co-workers (1960, 1961). The curve obtained in 100% acidic methanol (Figure 2, Methanol), has m' values of -8,800° at 234 mμ and +46,000° at 198 mμ, indicating the presence of 50-55% α-helix.

The optical rotation of the native β-lactoglobulins in the wavelength region below 240 mμ is not immediately interpretable. It is evident that this is the region which gives rise to the small changes in a_o observed between pH 2 and 8.5, since the variations in a_o parallel changes in the depth of the 230-240 mμ trough. At pH 11.2,

when the protein is denatured (a_o = -550), the new minimum around 208 mμ reflects the presence of random structure which also causes the 200 mμ maximum to be displaced to lower wavelengths (Blout et al., 1962). The structure responsible for the well-defined minimum at 232 mμ is not known.[3] In the native state the bimodal minimum between 228 and 240 mμ with a small maximum at 233 mμ indicates the presence of some ordered structure, probably non-α-helical (a possibility recently demonstrated by Litman and Schellman (1965)), although a small amount of α-helix (~ 8%) is not excluded, especially in view of the peak at 202 mμ. Finally, in relation to the present results, it is of interest to note that the infrared spectrum of an aqueous solution of β-lactoglobulin in the native state displays an amide I band with a maximum at 1,632 cm^{-1} (Timasheff and Susi, unpublished), a frequency normally associated with the β-structure of polypeptides (Miyazawa and Blout, 1961; Krimm, 1962), while denaturation results in a shift of this band to 1,643 cm^{-1}, i.e. to a position identical with that found in the "randomly coiled" protein, α_s-casein.

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[3]It is interesting to note that at this pH, analysis of the ORD data in terms of the Schechter and Blout (1964) relation gives a normal result, both A constants agreeing on ca. 18% α-helix, while in the native protein no such agreement exists (Timasheff and Kumosinski, unpublished).

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